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NOTES

Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry as an Alternative to 16S rRNA Gene Sequencing for Identification of Difficult-To-Identify Bacterial Strains▽†

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Conventional methods are sometimes insufficient to identify human bacterial pathogens, and alternative techniques, often molecular, are required. Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) identified with a valid score 45.9% of 410 clinical isolates from 207 different difficult-to-identify species having required 16S rRNA gene sequencing. MALDI-TOF MS might represent an alternative to 16S rRNA gene sequencing.

The technique matrix-assisted laser desorption ionization–time-of-flight mass spectrometry (MALDI-TOF MS) has recently been introduced in diagnostic microbiology laboratories and has been shown to be a promising method to replace conventional phenotypic identification for the majority of bacterial pathogens routinely found in clinical samples (1, 3, 8, 15, 17).

For a subset of bacterial isolates, conventional techniques which mainly rely on commercial biochemical kit tests fail to provide a definitive identification of these infrequent isolates. In those cases, alternative techniques are required. That could be an expanded battery of biochemical tests, high-performance liquid chromatography (HPLC), or sequencing of housekeeping bacterial genes such as 16S rRNA genes and comparing the obtained sequences to those in databases (4). Since 16S rRNA gene sequencing is both time-consuming and costly, we wanted to assess the potential of MALDI-TOF MS, which is based on the study of proteic profiles, to replace it.

We investigated a collection of 1,405 isolates on which 16S rRNA gene sequencing was performed in the last 8 years in our laboratory due to a lack of satisfactory identification by conventional methods such as Vitek2 or the API system (bioMérieux, Marcy l'Etoile, France). We selected for each species a maximum of 5 isolates. This yielded a total of 433 bacterial isolates representing 207 different species from 84 different genera. For 23 isolates, no growth could be obtained from frozen stock. A total of 410 isolates were consequently further studied. Acquisition of protein mass spectra of strains by MALDI-TOF MS was done on a Microflex LT instrument (Bruker Daltonics, Leipzig, Germany) using the FlexControl 3.0 software (Bruker Daltonics) after a formic acid-acetonitrile extraction step was performed as previously described (1). Automated data analysis of raw spectra was performed by the BioTyper automation 2.0 software (Bruker Daltonics), using a library of 3,290 spectra (database update of 2 September 2008) with default settings. According to the criteria proposed by the manufacturer, a result was considered valid at the species level whenever the score value attributed by BioTyper was $x \geq 2.0$, valid at the genus level when the score was $1.7 \leq x < 2$, and no reliable identification when the score was $x < 1.7$. 16S rRNA gene sequencing and identification were performed by standard methods (5).

For routine isolates, MALDI-TOF MS has shown overall correct identification levels often greater than 85% (1, 3, 8, 15, 17). In the present study, MALDI-TOF MS yielded a score of $x \geq 2.0$ for 204/410 (49.8%) of isolates and a score of $1.7 \leq x < 2$ for 73/204 (17.8%) of isolates. For the remaining 133/410 (32.4%) isolates, no reliable identification (score of $x < 1.7$) was obtained. Among the 204 isolates with a score of $x \geq 2.0$, 188/204 (92.2%) were concordant at the species level with 16S rRNA identification and 16/204 (7.8%) were discordant at the species level. For these 16 discordant results, complementary phenotypic tests were performed in order to arbitrate between the two identifications. The detailed description of these discordant results and the outcome of additional tests are presented in Table 1. These additional tests indicated that the identification by MALDI-TOF MS was correct for *Achromobacter ruhlandii*, *Lactobacillus rhamnosus*, *Ochrobactrum intermedium*, and *Pasteurella multocida*. In addition, we took advantage of an update of the BioTyper database (to version 3.1 of 7/5/2010, containing 3,740 spectra) during the revision process of the manuscript to perform a new MALDI-TOF MS identification of these isolates. Interestingly, three

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TABLE 1. Description of discordant results at the species level between the 16S rRNA gene identification ("gold standard") and MALDI-TOF MS among strains with a score of $x \geq 2$

16S rRNA gene identification (no. of isolates)	MALDI-TOF MS identification (no. of isolates)	Presence in MALDI-TOF MS database (no. of spectra)[a]	Commentary (reference)	Result upon additional testing
Achromobacter xylosoxidans (2)	*Achromobacter ruhlandii* (2)	Yes (4)	This discordance has been observed in a previous study (1).	Positive reduction of nitrate into nitrite indicates that the correct identification is *A. ruhlandii*. A new MALDI-TOF MS identification with the updated database still yields *A. ruhlandii*.[b]
Acinetobacter junii (1)	*Acinetobacter grimontii* (1)	Yes (2)	*A. grimontii* is a heterotypic synonym of *A. junii* (16).	A new MALDI-TOF MS identification with the updated database still yields *A. grimontii*.[b]
Actinobacillus hominis (1)	*Actinobacillus suis* (1)	No	*A. suis* is mainly a pig pathogen and is rarely involved in human infections (10).	Lack of growth on MacConkey agar and α-haemolysis on blood agar indicates that the correct identification is *A. hominis*. A new MALDI-TOF MS identification with the updated database yields *A. equuli*.[b]
Aerococcus sanguinicola (1)	*Aerococcus urinae* (1)	Yes (1)	*A. urinae* and *A. sanguinicola* are difficult to identify by conventional phenotypic methods (2).	Positive pyrrolidonyl aminopeptidase activity indicates that the correct identification is *A. sanguinicola*. A new MALDI-TOF MS identification with the updated database now yields *A. sanguinicola*.[b]
Corynebacterium aurimucosum (1)	*Corynebacterium minutissimum* (1)	Yes (2)	*C. aurimucosum* is a recently described *Corynebacterium* species (18).	Positive hippurate hydrolysis indicates that the correct identification is *C. aurimucosum*. A new MALDI-TOF MS identification with the updated database now yields *C. aurimucosum*.[b]
Corynebacterium glucuronolyticum (3)	*Corynebacterium seminale* (3)	Yes (1)	*C. seminale* is a heterotypic synonym of C. *glucuronolyticum* (7).	A new MALDI-TOF MS identification with the updated database now yields *C. glucuronolyticum*.[b]
Dysgonomonas capnocytophagoides (1)	*Dysgonomonas gadei* (1)	No	Dysgonomonas are rare human pathogens which have been recently described (11).	Negative catalase reaction indicates that the correct identification is *D. capnocytophagoides*. A new MALDI-TOF MS identification with the updated database still yields *D. gadei*.[b]
Enterococcus saccharolyticus (1)	*Enterococcus phoeniculicola* (1)	Yes (1)	*E. phoeniculicola* has been isolated from a bird; it is not known as a human pathogen (12).	Positive mobility, a vancomycin MIC of 6, and a new biochemical identification by Vitek2 indicates that the isolate is *E. casseliflavus*. A new MALDI-TOF MS identification with the updated database now yields *E. casseliflavus*.[b]
Kingella kingae (1)	*Kingella* sp. (1)	Yes (3)	The presence of reference spectra of strains identified only to the genus level is misleading and precludes definitive identification to the species level; such spectra should be discarded from the database.	A new MALDI-TOF MS identification with the updated database now yields *K. kingae*.[b]

Lactobacillus fermentum (1)	*Lactobacillus rhamnosus* (1)	Yes (3)	Conventional methods often fail to identify lactobacilli at the species level (13).	An API 50 CH carbohydrate strip indicates that the correct identification is *L. rhamnosus*. A new MALDI-TOF MS identification with the updated database still yields *L. rhamnosus*.[b]
Ochrobactrum anthropi (1)	*Ochrobactrum intermedium* (1)	Yes (3)	*O. anthropi* and *O. intermedium* are phenotypically and genetically closely related pathogens (14).	Resistance to colistin indicates that the correct identification is *O. intermedium*. A new MALDI-TOF MS identification with the updated database still yields *O. intermedium*.[b]
Pandoraea sputorum (1)	*Pandoraea pnomenusa* (1)	No	*Pandoraea* species are difficult to differentiate by conventional and molecular methods (6).	A negative urease test and lack of growth at 42°C indicates that the correct identification is *P. sputorum*. A new MALDI-TOF MS identification with the updated database still yields *P. pnomenusa*.[b]
Pasteurella pneumotropica (1)	*Pasteurella multocida* (1)	No	*P. pneumotropica* is a rare human pathogen, difficult to identify by conventional methods (9).	A negative urease test, lack of growth on MacConkey agar, and coccoid Gram-negative staining indicate that the correct identification is *P. multocida*. A new MALDI-TOF MS identification with the updated database still yields *P. multocida*.[b]

[a] Presence of the 16S rRNA gene-identified species in the MALDI-TOF MS database. The number of different reference spectra per species is shown in parentheses.
[b] Database updated during the revision process to version 3.1 (5 July 2010), which contains 3,740 spectra.

MALDI-TOF MS identifications became concordant with 16S rRNA gene results (*Aerococcus sanguinicola*, *Corynebacterium aurimucosum*, and *Corynebacterium glucuronolyticum*). One MALDI identification of *Enterococcus phoeniculicola* changed for *Enterococcus casseliflavus*, which turned out to be the species now identified by additional conventional methods (instead of the original *Enterococcus saccharolyticus* identified by 16S rRNA gene). Finally, the identification of *Kingella* sp. from the original database has been replaced by a concordant *Kingella kingae* identification (Table 1). Among the 73 isolates with a score of $1.7 \leq x < 2$, 66/73 (90.4%) were concordant at the species level and 7/73 (9.6%) were concordant at the genus level. No misidentifications at the genus level were observed in the study. Hence, 254/410 (62%) strains were concordant at the species level between 16S rRNA gene sequencing and MALDI-TOF MS. However, since a score of $x \geq 2.0$ is recommended for the clinical microbiologist to consider accurate an identification at the species level, only 188/410 (45.9%) of 16S rRNA gene sequencing identifications could have been replaced by MALDI-TOF MS.

A summary of the results is presented in Fig. 1, and detailed results are shown in Table S1 in the supplemental material. Of note, known heterotypic synonyms of basic local alignment search tool results of 16S rRNA gene sequencing or identified in a previous study performed in our laboratory (1) were corrected prior to analysis.

For 133 strains (corresponding to 85 different species; see Table S1 in the supplemental material), failure to obtain an identification (score of $x < 1.7$) can be attributed to two causes. The first cause is the absence of an adequate reference spectrum in the BioTyper database. This was the case for 78/133 (58.6%) of this subset of isolates. The second cause is a failure to obtain a sufficient protein signal in order to build a spectrum that can be compared to the BioTyper database. This was the case for 55/133 (41.4%) isolates. Failure to obtain a spectrum can be explained either because of the structural properties of the cell wall of some bacteria (Gram-positive bacilli being a prototypical example) or fastidious growth of some isolates which yield only small amounts of colonies that can be harvested for protein extraction purposes. As an example, a clear genus trend can be seen for *Actinomyces*, *Gemella*, *Nocardia*, and *Streptomyces* species, which are either not identified or only poorly identified by MALDI-TOF MS in spite of the presence of some reference spectra (see Table S1 in the supplemental material).

Regarding time to results and cost-effectiveness, whereas a MALDI-TOF MS identification is performed in minutes and costs a few U.S. dollars (3, 15), 16S rRNA gene sequencing is time-consuming in terms of both reagents and technicians. Including consumables, salaries, and depreciation of the apparatuses, a 16S rRNA identification performed in our laboratory typically costs ca. 100 U.S. dollars and is available in 48 h.

In conclusion, our study shows that MALDI-TOF MS has the potential to reduce the need for molecular identification techniques such as 16S rRNA gene sequencing and might replace these time-consuming and expensive techniques for the majority of difficult-to-identify isolates in the clinical microbiology laboratory. Because the percentage and type of strains for which sequencing is required differ among

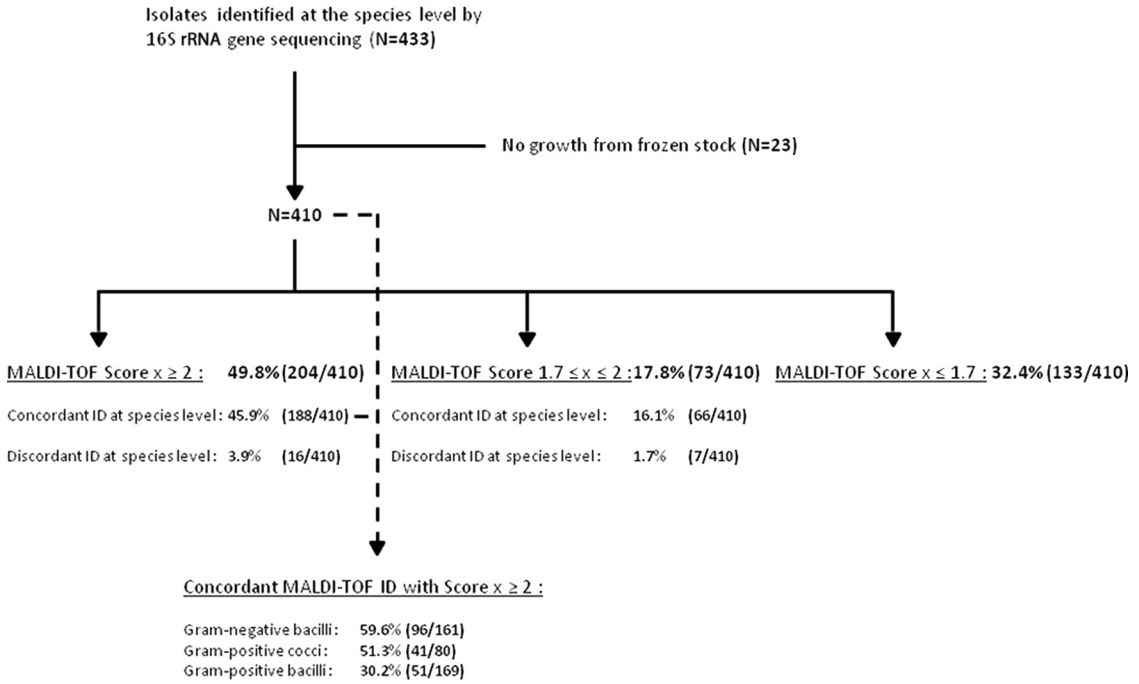

FIG. 1. Identification (ID) yield of MALDI-TOF MS analysis for 410 bacterial isolates identified at the species level by 16S rRNA gene sequencing.

laboratories according to local epidemiology and identification-level requirements, the number of strains that can be identified by MALDI-TOF MS instead of molecular methods will vary. As well, further expansion of the database of the instrument and optimization of extraction protocols for difficult-to-treat samples will undoubtedly increase the accuracy of identification by the MALDI-TOF MS and the diversity of species that might be efficiently identified by this promising approach.

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